

#4

I hereby certify that this correspondence is being deposited with the United States Postal Service as first-class mail in an envelope addressed to:

PATENT
Attorney Docket No.: 020444-000710US

U.S. Patent and Trademark Office
Box SEQUENCE
P.O. Box 2327
Arlington, VA 22202

On 4/5/02

TOWNSEND and TOWNSEND and CREW LLP

By: Jennifer K. Handi

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

SCHNEIDER *et al.*

Application No.: 10/035,349

Filed: October 19, 2001

For: MASS DEFECT LABELING FOR
THE DETERMINATION OF
OLIGOMER SEQUENCES

Examiner: Not yet assigned

Art Unit: 1645

COMMUNICATION UNDER

37 C.F.R. §§ 1.821-1.825

AND

PRELIMINARY AMENDMENT

U.S. Patent and Trademark Office
Box SEQUENCE
P.O. Box 2327
Arlington, VA 22202

Sir:

In response to the request to comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures, 37 C.F.R. §§ 1.821-1.825, that accompanied the Notice to File Missing Parts of Nonprovisional Application mailed February 5, 2002, Applicants submit herewith the required paper copy and computer readable copy of the Sequence Listing. Please amend the specification in adherence with 37 C.F.R. §§ 1.821-1.825 as follows.

2005 F40 5465601

In the Specification:

Please replace the paragraph beginning at page 14, line 3, with the following:

--**FIG. 17** shows the result of using "sequencer" code to determine the sequence of the first five residues in 5-Br-3-PAA-labeled myoglobin (SEQ ID NOS:1 and 2).--

Please replace the paragraph beginning at page 14, line 9, with the following:

--**FIG. 19** illustrates an exemplary sequencing technique using the methods described by Sanger in combination with the labeling strategy provided herein (SEQ ID NOS:3-7).--

Please replace the paragraph beginning at page 14, line 13, with the following:

--**FIG. 21** illustrates a ddA*/ddG* mass spectrum (SEQ ID NO:5) (see Example 18).--

Please replace the paragraph beginning at page 14, line 14, with the following:

--**FIG. 22** illustrates a ddT*/ddC* mass spectrum (SEQ ID NOS:5 and 6) (see Example 18).--

Please replace the paragraph beginning at page 78, line 20, with the following:

--The raw data used to generate the mass spectrum from Example 5 is exported in ASCII format from the data acquisition system. The natural period of the chemical noise is determined from this raw data using the "deconvolver" code shown in

20540 "0455001

the appendix and is determined to be 1.000575 amu. Using this natural period the spectrum is baselined (output file *.bsl) to correct for instrument error, which is always positive in MS (Figure 16). Baselining means that the minimum data value in each 1.000575 amu block of data is adjusted to zero by subtracting through every data point in the block of data. The baselined data file is subsequently processed with the "betafactor" as a way to qualify mass defect (Br-containing) peaks, which should always have a matching [⁸¹Br] peak 1.997954 amu upstream from the [⁷⁹Br] peak (Figure 16). The resulting *.bfc file is then processed through the "sequencer" code (see, co-pending application filed October 19, 2001, entitled "Methods for Determining Protein and Peptide Terminal Sequences" Atty Docket No. 05265.P001, the disclosure of which is incorporated herein by reference), with the true N-terminal myoglobin sequence (5-Br-3-PAA-GLSDGE; SEQ ID NO:1) being the top ranked solution through the first four residues. In this example the "sequencer" code was limited search for the first charge state of b-ions.--

Please replace the paragraph beginning at page 79, line 3, with the following:

--When the "sequencer" code is run to determine the sequence of the first five residues, the sequence GLSDW (SEQ ID NO:8), which yields a theoretical mass of 756.1993 overlaps (Figure 17) the peak corresponding to the mass defect position of the sixth residue of the true sequence (GLSDGE (SEQ ID NO:9) at 756.1840). This results in GLSDW (SEQ ID NO:8) being the top ranked sequence at five residues. However, when "sequencer" is run through six residues the true sequence GLSDGE (SEQ ID NO:9) becomes top ranked again because GLSDW (SEQ ID NO:8) fails to propagate a competing sequence at the sixth residue. This shows the advantage of a cumulative probability algorithm.--

Please replace the paragraph beginning at page 80, line 13, with the following:

--Briefly, an M13 plasmid carrying a cloned unknown DNA sequence (e.g., d(GTTACAGGAAAT) (SEQ ID NO:10)) is initially hybridized with an M13 origin of replication primer (3'-d(AGTCACGACGACGTTGT)rA-5'; SEQ ID NO:4) (d(AGTCACGACGACGTTGT)rA) that is labeled at the 5' 3' end with rA to make the primer selectively cleavable by RNase (Integrated DNA Technologies, Inc., Coralville, Iowa). The reaction volume is divided in half and transferred to two tubes. In one tube, polymerase, dNTPs, dGTP, and mass-defect-labeled ddATP* (see Figure 20a) and ddGTP* (see Figure 20b) are added. To the other tube, polymerase, dNTPs, and mass-defect-labeled ddTTP* (see Figure 20c) and ddCTP* (see Figure 20d) are added. The modified ddNTPs shown in Figure 20 are exemplary and are prepared according to standard procedures (see, Kricka, L.J., NONISOTOPIC DNA PROBE TECHNIQUES, Academic Press, New York (1992); Keller, G.H. and Manak, M.M., DNA PROBES, Stockton, New York (1989)). Many other modified ddNTPs are useful which contain purine and pyrimidine bases derivatized with mass defect label moieties and separated by a large assortment of crosslinkers with different lengths and/or compositions. DNA replication and chain extension is initiated by incubation at 37°C. Mass ladders are produced by chain termination with the ddNTPs. A denaturation and cleavage step with RNase at the end of the reaction removes the chain-terminated product from the template and frees the primer that can be selectively removed by hybridization. The DNA fragments are dissolved in a mass spectrometer-compatible buffer and flown in an ESI-TOF mass spectrometer in negative ion mode. The peaks corresponding to a series of multiply-charged ions for each fragment are deconvolved using standard algorithms supplied by the instrument manufacturer (Applied Biosystems) to generate spectra containing only the zero-charge masses. The zero-charge spectra are subsequently centroided also using the instrument supplier's algorithms.--

20549 0430
20549 0430

Please replace the paragraph beginning at page 81, line 3, with the following:

--The mass spectral data are analyzed as follows. The spectrum from the ddA*- and ddG*-containing sample is deconvolved and chemical noise is eliminated, leaving only peaks that have incorporated bromine or iodine atoms (Figure 21). The spectrum from the ddT*- and ddC*-containing sample is similarly treated (Figure 22). Looking at both deconvolved spectra, the highest mass fragment is found (4114.733) in the ddA*/ddG* spectrum (Figure 21). This fragment can also be identified as the fragment that contains an iodine mass element as there is no isotopic pair; therefore, the last nucleotide in the "unknown" sequence is A. The mass fragment with the next lower mass is a doublet at 3695.611 and 3697.609 which is found in the ddT*/ddC* spectrum (Figure 22). The doublet indicates incorporation of a bromine atom, and, therefore, the next nucleotide in the sequence is T. This process is repeated until the last peak is found, in this case, a singlet peak at 748.1850 in the ddT*/ddC* spectrum corresponding, therefore, to C. Thus, the sequence ATTCCTGTAAAC (SEQ ID NO:11) is determined, and when inverted and the nucleotide complements are substituted, the "unknown" sequence GTTACAGGAAAT (SEQ ID NO:10) is determined.--

Please replace the paragraph beginning at page 82, line 3, with the following:

--The true ubiquitin N-terminal sequence (MQIFVK (SEQ ID NO:12), obtained from GenBank) was correctly determined when "sequencer" was run to two, three, and four residues. The correct sequence ranked second out of 19 competing possibilities at the first residue. The correct sequence was also ranked second (to MQIFR (SEQ ID NO:13)) at the fifth residue.--

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1 to 4, at the end of the application.

205740" 64E5E00T

REMARKS

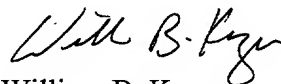
Applicants request entry of this amendment in adherence with 37 C.F.R. §§1.821 to 1.825. This amendment is accompanied by a floppy disk containing the above named sequences, SEQ ID NOS:1-13, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

Attached hereto is a marked-up version of the changes made to the Specification by the current Amendment. The attached pages are captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



William B. Kezer
Reg. No. 37,369

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
WBK:dmw

205740 STEELED

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 3 of page 14 has been amended as follows:

FIG. 17 shows the result of using "sequencer" code to determine the sequence of the first five residues in 5-Br-3-PAA-labeled myoglobin (SEQ ID NOS:1 and 2).

Paragraph beginning at line 9 of page 14 has been amended as follows:

FIG. 19 illustrates an exemplary sequencing technique using the methods described by Sanger in combination with the labeling strategy provided herein (SEQ ID NOS:3-7).

Paragraph beginning at line 13 of page 14 has been amended as follows:

FIG. 21 illustrates a ddA*/ddG* mass spectrum (SEQ ID NO:5) (see Example 18).

Paragraph beginning at line 14 of page 14 has been amended as follows:

FIG. 22 illustrates a ddT*/ddC* mass spectrum (SEQ ID NOS:5 and 6) (see Example 18).

205140 64500T

Paragraph beginning at line 20 of page 78 has been amended as follows:

The raw data used to generate the mass spectrum from Example 5 is exported in ASCII format from the data acquisition system. The natural period of the chemical noise is determined from this raw data using the "deconvolver" code shown in the appendix and is determined to be 1.000575 amu. Using this natural period the spectrum is baselined (output file *.bsl) to correct for instrument error, which is always positive in MS (Figure 16). Baselining means that the minimum data value in each 1.000575 amu block of data is adjusted to zero by subtracting through every data point in the block of data. The baselined data file is subsequently processed with the "betafactor" as a way to qualify mass defect (Br-containing) peaks, which should always have a matching [⁸¹Br] peak 1.997954 amu upstream from the [⁷⁹Br] peak (Figure 16). The resulting *.bfc file is then processed through the "sequencer" code (see, co-pending application filed October 19, 2001, entitled "Methods for Determining Protein and Peptide Terminal Sequences" Atty Docket No. 05265.P001, the disclosure of which is incorporated herein by reference), with the true N-terminal myoglobin sequence (5-Br-3-PAA-GLSDGE; SEQ ID NO:1) being the top ranked solution through the first four residues. In this example the "sequencer" code was limited search for the first charge state of b-ions.

Paragraph beginning at line 3 of page 79 has been amended as follows:

When the "sequencer" code is run to determine the sequence of the first five residues, the sequence GLSDW (SEQ ID NO:8), which yields a theoretical mass of 756.1993 overlaps (Figure 17) the peak corresponding to the mass defect position of the sixth residue of the true sequence (GLSDGE (SEQ ID NO:9) at 756.1840). This results in GLSDW (SEQ ID NO:8) being the top ranked sequence at five residues. However, when "sequencer" is run through six residues the true sequence GLSDGE (SEQ ID NO:9) becomes top ranked again because GLSDW (SEQ ID NO:8) fails to propagate a

competing sequence at the sixth residue. This shows the advantage of a cumulative probability algorithm.

Paragraph beginning at line 13 of page 80 has been amended as follows:

Briefly, an M13 plasmid carrying a cloned unknown DNA sequence (e.g., d(GTTACAGGAAAT) (SEQ ID NO:10)) is initially hybridized with an M13 origin of replication primer (3'-d(AGTCACGACGACGTTGT)rA-5'; SEQ ID NO:4) (d(AGTCACGACGACGTTGT)rA) that is labeled at the 5' end with rA to make the primer selectively cleavable by RNase (Integrated DNA Technologies, Inc., Coralville, Iowa). The reaction volume is divided in half and transferred to two tubes. In one tube, polymerase, dNTPs, dGTP, and mass-defect-labeled ddATP* (see Figure 20a) and ddGTP* (see Figure 20b) are added. To the other tube, polymerase, dNTPs, and mass-defect-labeled ddTTP* (see Figure 20c) and ddCTP* (see Figure 20d) are added. The modified ddNTPs shown in Figure 20 are exemplary and are prepared according to standard procedures (see, Kricka, L.J., NONISOTOPIC DNA PROBE TECHNIQUES, Academic Press, New York (1992); Keller, G.H. and Manak, M.M., DNA PROBES, Stockton, New York (1989)). Many other modified ddNTPs are useful which contain purine and pyrimidine bases derivatized with mass defect label moieties and separated by a large assortment of crosslinkers with different lengths and/or compositions. DNA replication and chain extension is initiated by incubation at 37°C. Mass ladders are produced by chain termination with the ddNTPs. A denaturation and cleavage step with RNase at the end of the reaction removes the chain-terminated product from the template and frees the primer that can be selectively removed by hybridization. The DNA fragments are dissolved in a mass spectrometer-compatible buffer and flown in an ESI-TOF mass spectrometer in negative ion mode. The peaks corresponding to a series of multiply-charged ions for each fragment are deconvolved using standard algorithms supplied by the instrument manufacturer (Applied Biosystems) to generate spectra

containing only the zero-charge masses. The zero-charge spectra are subsequently centroided also using the instrument supplier's algorithms.

Paragraph beginning at line 3 of page 81 has been amended as follows:

The mass spectral data are analyzed as follows. The spectrum from the ddA*- and ddG*-containing sample is deconvolved and chemical noise is eliminated, leaving only peaks that have incorporated bromine or iodine atoms (Figure 21). The spectrum from the ddT*- and ddC*-containing sample is similarly treated (Figure 22). Looking at both deconvolved spectra, the highest mass fragment is found (4114.733) in the ddA*/ddG* spectrum (Figure 21). This fragment can also be identified as the fragment that contains an iodine mass element as there is no isotopic pair; therefore, the last nucleotide in the "unknown" sequence is A. The mass fragment with the next lower mass is a doublet at 3695.611 and 3697.609 which is found in the ddT*/ddC* spectrum (Figure 22). The doublet indicates incorporation of a bromine atom, and, therefore, the next nucleotide in the sequence is T. This process is repeated until the last peak is found, in this case, a singlet peak at 748.1850 in the ddT*/ddC* spectrum corresponding, therefore, to C. Thus, the sequence ATTCCTGTAAC (SEQ ID NO:11) is determined, and when inverted and the nucleotide complements are substituted, the "unknown" sequence GTTACAGGAAAT (SEQ ID NO:10) is determined.

Paragraph beginning at line 3 of page 82 has been amended as follows:

The true ubiquitin N-terminal sequence (MQIFVK (SEQ ID NO:12), obtained from GenBank) was correctly determined when "sequencer" was run to two, three, and four residues. The correct sequence ranked second out of 19 competing possibilities at the first residue. The correct sequence was also ranked second (to MQIFR (SEQ ID NO:13)) at the fifth residue.